CRYOPRESERVATION STRATEGIES FOR FARM ANIMAL GENETIC RESOURCES IN EUROPE

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Abstract

European countries have developed national strategies and action plans implementing the Global Plan of Action for animal genetic resources. National action plans include development and implementation of cryopreservation strategies for animal genetic resources. Although some cross-border collaboration exists in Europe, cryopreservation programmes are national responsibilities and generally organised at the national level. National cryopreservation programs have different specific objectives and involvement of stakeholders and institutions varies. National experts indicate that there is a need for further development of national frameworks for cryopreservation and for exchange of state of the art knowledge and experiences between countries. The aim of the European Regional Focal Point is to support development of national conservation programmes and to enhance stronger international collaboration through development of a European cryopreservation strategy.

Keywords: cryopreservation, European collaboration, animal genetic resources, local breeds

Introduction

In 2007, the international community has adopted the Global Plan of Action for Animal Genetic Resources (FAO, 2007b). European countries are currently implementing the Global Plan. Already since the 1970s and 1980s many European countries have been paying attention to local breeds and have saved many of them from total extinction and contributed actively to assessing the State of the World's Animal Genetic Resources (FAO, 2007a). European countries are individually and in collaboration carrying out active work on animal genetic resources (Mäki-Tanila and Hiemstra, 2010).

During the 2nd half of the 20th century, specialized livestock breeds were developed in Europe. High-input high-output breeds and the associated (intensive) production systems were widely promoted and distributed within and outside Europe. At the same time population sizes of many native breeds have decreased dramatically. About 30% of the European breeds are reported "at risk" (FAO, 2007a). Although there are still threats to native breeds in Europe, luckily most of the breeds survived. There has been growing awareness about the values of native breeds among a variety of stakeholders, that is also reflected in promoting conservation and sustainable use of genetic resources in EU and national policies.

In situ conservation is often regarded as the preferred conservation method for maintaining variation in local breeds, because it ensures that a breed is maintained in a dynamic state. This may be true when the dynamics of a breed are characterized by slow and balanced adaptation to (changing) conditions, but reality is different. Many factors can positively or negatively influence breed dynamics (Hiemstra et al., 2010). When *in situ* conservation programmes are not properly planned, breeds may be threatened. Therefore both medium and long term conservation of livestock genetic diversity will greatly benefit from a complementary cryopreservation (*ex situ)* strategy. Cryopreservation is the collection and deep-freezing of semen, ova, embryos or tissues which may be used for future breeding or regenerating animals (FAO, 2010). Cryopreservation of germplasm is not only a very useful tool to maintain genetic diversity within breeds and to support the genetic management of breeds, but also, in the worst case, the cryopreserved germplasm could be used to re-establish a breed or population.

European countries have developed national strategies and action plans to implement the Global Plan of Action for Animal Genetic Resources (FAO, 2007b). Strategies Priority area three of the Global Plan of Action ("Conservation") includes an *ex situ* conservation component in order to preserve genetic diversity and integrity for the benefit of current and future generations. In many countries gene bank

collections have been established, however national programmes are quite different in terms of stakeholder involvement and institutional framework (e.g. Pizzi et al., 2010).

The aim of this paper is to discuss the main issues for further development of cryopreservation strategies in Europe and to describe the current state of cryopreservation and recent initiatives.

State of cryopreservation in Europe

According to the State of the World's Animal Genetic Resources report (FAO, 2007a) most *in vitro* conservation programmes in Europe are found in the western and central part of the region. Several gene banks were recently founded and there is a need for further development. In many cases cryopreservation is restricted to the storage of semen from a limited number of breeds. A few countries have gene banks preserving semen of all main species. The report also mentioned that – despite the presence of rich AnGR diversity in combination with real threats (such as political instability) - *in vitro* conservation programmes are largely absent in the eastern parts of the European region. Furthermore, ownership and access, information and documentation, optimization of the core collection and the ratio between gametes and embryos were mentioned as imported issues for further development.

More recently, FAO implemented a survey (Boettcher and Akin, 2010), including various matters related to AnGR conservation, including multinational gene banks. Results of the survey show that many countries practise AnGR conservation, with *in situ* programmes being the most common. The number of cryoconservation programmes was about half the number of *in situ* programmes for most livestock species. Fully operational gene banks were reported in about 20 percent of the countries, and plans for a gene bank within 5 years were indicated in an additional 50 percent of the countries. Lack of financial support and low priority in national livestock policy were the most commonly cited obstacles for gene banking. Comparing different regions, this survey indicated that cryoconservation programmes are most common in North America, followed by Europe, and then Asia, the Near East, Africa and Latin America.

In addition to other comparisons of national cryopreservation programmes (e.g. Blackburn, 2004; Danchin Burge et al., 2006; Danchin-Burge et al., 2011), a detailed survey was carried out within the European Commission co-funded project EURECA (www.regionalcattlebreeds.eu) to compare cryopreservation activities and policies for cattle breeds in Finland, France, Italy and the Netherlands. The purpose of the survey was to detect similarities and differences between these four countries, to compare the countries' strategies and with the international Guidelines, and to formulate recommendations for initiating or strengthening cryopreservation programmes (Pizzi et al. (2010).

Pizzi et al. (2010) showed that although overall objectives were rather similar for the four European national cryopreservation programmes studied, the programmes have developed differently. In particular the institutional framework and stakeholders responsibilities and involvement are different. All countries expressed the need for national or public funding for national cryopreservation programmes with long term objectives. However, the close involvement of breeders of local breeds, breed associations and AI centres in linking the cryopreservation schemes with routine AI operations was considered as the most important factor for the development of efficient cryopreservation programmes.
The most commonly cryopreserved genetic material of European local cattle breeds in the four countries is semen (Pizzi et al., 2010). Another European wide survey (Hiemstra et al. 2010) also showed that for 93 out of 108 breeds only semen is stored, whereas only in 26 out of 108 breed both semen and embryos are stored.
Sampling strategies and selection of donors vary between the four countries surveyed by Pizzi et al. (2010). Two main sampling strategies were observed: 1) few bulls with many doses are stored, and 2) semen of as many bulls, but fewer straws is collected. For example, in the Netherlands and Italy, the majority of bulls have less than 200 doses stored as genetic reserve. Conversely, in France and Finland, almost 50% of the bulls have more than 1000 doses collected. In France, Finland and the Netherlands, almost a quarter of the bulls stored were born before 1980, which is an important genetic reserve for the particular breeds.
Depending on the national veterinary regulations, semen for the national gene banks has been collected on AI centres with high veterinary standards, but there is also semen collection for the gene

bank “on farm”. Furthermore, experts involved in the cryopreservation programmes of Finland, France, Italy and the Netherlands were asked to identify and rank the key factors affecting the state of AnGR cryopreservation in their country. Internal factors (Strengths and Weaknesses) and external factors (Opportunities and Threats) were identified and used to assess the national situation regarding cryopreservation and to identify and design new or better policies at national or European levels. Pizzi et al. (2010) and Hiemstra et al. (2010) finally concluded that there is a need for countries to (further) develop and implement national regulatory frameworks for cryopreservation.

European Regional Focal Point

The main responsibility for implementing the Global Plan of Action rests with national governments. The implementation of the GPA by individual countries is facilitated by, and benefits from, the existence of international networks of NCs. Regional Focal Points, such as the European Regional Focal Point for Animal Genetic Resources (ERFP) play a vital and important role in building collaborative partnerships, in coordinating regional management efforts in animal genetic resources, in further developing information sharing and enhancing technical cooperation, training and research (www.rfp-europe.org). The ERFP was initiated in 1998 and became formally operational in 2001 as the European part of FAO's global coordination structure for animal genetic resources. Over the years, the collaboration and coordination facilitated by the ERFP in Europe has developed successfully. In 2010 the General Assembly of the ERFP adopted a new Multi Year Programme of Work (MYPOW) for the period 2010-2014.

ERFP has also established close working relationship with international NGOs such as Rare Breeds International (RBI), The SAVE Foundation (Safeguard for Agricultural Varieties in Europe), Danubian Alliance for Conservation of Genes in Animal Species (DAGENE) or the European Forum of Farm Animal Breeding (EFFAB). For all the scientific aspects, it receives help from the European Association of Animal Production Working Group on Animal Genetic Resources (EAAP WG-AGR). The ERFP does not create new structures but relies as far as possible on existing functional structures in the different countries.

Among other objectives the ERFP aims to support the *in situ* and *ex situ* conservation and sustainable use of AnGR in European countries. The ERFP works with existing operational structures in countries and seeks partnerships and collaboration with existing organisations. Already in 2003 ERFP published Guidelines for the Constitution of National Cryopreservation Programmes for Farm Animals (ERFP, 2003) and organised a workshop on this topic (Planchenault, 2003). The ERFP also significantly supported the development, review, testing and validations of the Draft Technical FAO Guidelines for the Cryoconservation of AnGR (FAO, 2010) through various workshops involving more than 120 scientists, technicians and decision makers.

Working Group *Ex Situ* Conservation of ERFP

The Assembly of National Coordinators of the ERFP established several Working Groups and Task Forces (www.rfp-europe.org). The *Ex Situ* Conservation Working Group is one of the ERFP Working Groups that works on a permanent basis on long term tasks. Members of the Working Group are experts in the respective task and are nominated by their National Coordinator. The general aim of the Working Group is to regularly exchange experiences and knowledge between European countries in order to support the establishment, further development, efficiency and effectivity of national gene banks for farm animal genetic resources.

For the period 2011-2013 the Ex Situ Working Group of ERFP identified a number of high piority topics for the next two or three years, including i) prioritization – genetic aspects, ii) development of national legal and institutional frameworks, iii) maintenance and development of the CryoWeb database, and iv) development of a European gene bank strategy vision paper. To further develop national gene banks, there is a need to further exchange experience and knowledge between European countries. Moreover, the ERFP will initiate further discussions about a European strategy or European vision on *ex situ* conservation of farm animal genetic resources.

Prioritization and sampling strategies

In order to capture maximum genetic diversity in a gene bank, and because of limited availability of funding, prioritization of breeds, animals and type of germplasm/tissue for conservation will remain a relevant issue. Availability of new technologies (e.g. genomics, cryobiology, reproductive technology) may affect prioritization and sampling strategies. There is for example a need to further improve methods for prioritisation for conservation that take account of new genomic measurement and simultaneously exploit phenotypic, farming-system, socio-economic and geographic information. National cryopreservation programmes should use and implement state of the art knowledge and best practices for the selection of donor animals for conservation and to decide about the type of genetic material (semen, embryo, oocyte, tissue) to be stored.

According to the ERFP and FAO Guidelines (ERFP, 2003; FAO, 2010), one common purpose of a germplasm repository is to provide the possibility of recreating breeds or breeding lines in case they are lost as a consequence of a calamity. Storage of germplasm for this purpose would typically be long-term storage, without frequent use of the stored material and without the need of regular updating of the collection. A second way to make use of gene bank resources is to support *in vivo* conservation. Additionally, gene bank resources may be used as a back-up in case genetic problems occur. Finally, gene banks can serve as the primary source of material for national scientists performing DNA research.

The last decades have seen increasing possibilities for gene banking as a result of advances in cryobiology and reproductive technology. Semen and embryos can be obtained, cryopreserved, and used for most species of farm animals. Although embryos have an advantage over semen in breed reconstitution, cryopreservation of embryos has been limited to a smaller number of farm animal species. An advantage of cryopreservation of oocytes over embryos is that through in vitro fertilization (IVF) desired matings can be selected at the time of thawing, rather than at the time of freezing. However techniques for freezing and thawing of oocytes still require more development. The banking of somatic cells on the other hand requires only the collection and direct freezing of a piece of tissue. This would become an attractive strategy for cryoconservation of AnGR, if production of live animals from somatic cells through Somatic Cell Nuclear Transfer (SCNT) becomes efficient, safe, reliable and ethically acceptable.

Future prospects for cryoconservation may benefit from breakthroughs in cryopreservation and generation of offspring from cryopreserved i) (parts of) ovaries, ii) Embryonic Stem Cells (ESC), iii) spermatogonial cells, iv) primordial germ cells or v) through parthenogenetic embryo production.

Legal framework

As said before, national cryopreservation programmes vary in institutional and legal frameworks. No single particular system of organization and institutions will be ideal for all situations and countries. The optimal system will depend on a wide variety of factors, including the types of existing infrastructure and related institutions, technical capacity of personnel, species of interest, stakeholders, and level of government versus private support. According to the FAO Guidelines on Cryoconservation (FAO, 2010), evaluation of the major institutions and stakeholders, their goals and their capacity to contribute to conservation programmes will be an essential step in the initial phases of the development cryoconservation strategies. Establishment of linkages among institutions will be critical to maximize efficiency. Collaboration with breed societies, the private sector and individual owners of breeding animals is crucial. Collaboration with artificial insemination (AI) centres for example is usually highly beneficial, whereas these centres will have both the technical capacity and the infrastructure for collection, freezing and storage of germplasm. Breed associations such as co-operative breeding and herd-book associations may see it as their responsibility to maintain breeds. As an organization they are clearly interested in the long-term well-being of the breeds and may organize and financially support cryoconservation activities.

The *Ex Situ* Working Group of ERFP noted that many countries want to further develop or refine their national legal and policy framework for cryopreservation activities. There are already substantial gene bank collections (at different sites) but a clear legal framework is often lacking. Members of the Working Group indicated that exchange of experiences, legal framework documentation, protocols, contracts, access rules and other information would be very helpful.

Maintenance and development of the CryoWeb database

Proper documentation of gene bank collections is crucial for the management of gene bank collections. Cofunded by the European Commission, the database tool CryoWeb was developed and implemented. For maintenance and further development of the CryoWeb database tool it is important to establish a CryoWeb user group and to link national databases at European level.

Development of a European gene bank strategy for AnGR

In the State of the World's AnGR report (FAO, 2007a) it was recommended that all countries should have their own or shared gene banks that contain cryopreserved material of their locally developed breeds and lines. Because many transboundary breeds exist, coordination between countries is required. Cooperation would be facilitated if national and regional gene banks operate under internationally agreed protocols
Hiemstra et al. (2010) recommended that countries should (further) develop cryopreservation activities within the framework of a national programme for AnGR. Besides the use of national cryopreserved stocks to support genetic management of local breeds, transnational cooperation should be encouraged to avoid duplicates in long-term cryoreserve of transboundary breeds and in order to make optimal use of limited funds.
Boettcher and Akin (2010) reported that very few multinational gene banks exist, but interest in such activities was high. Aversion to multi-country gene banks was noted in only about 10 percent of countries. Among the factors contributing to the paucity of multi-country AnGR gene banks are a lack of funding, regulations on international exchange of genetic material and a lack of consensus on procedures for the operation of gene banks. Relative to other regions, the greatest level of willingness and capacity to collect germplasm for multinational gene bank purposes was found in Europe.

Where plant genetic resources conservation is strongly dependent of a multi-lateral system of international seed banks (including a security back up at Svalbaard repository), such a framework for international collaboration in conservation for animal genetic resources, does not exist. However, in order to increase cost-efficiency and to reduce duplications between national European gene bank, there may also be a clear advantage in organising conservation of AnGR across countries in Europe (and possibly other regions). Danchin-Burge et al. (2011) compared for example French, Dutch and US Holstein Friesian semen collections. They first concluded that the national cryobanks captured substantial amounts of genetic diversity, compared with the current population, and the comparison showed to what extent the US, French and Dutch collections were genetically similar.
It is important to further elaborate collaboration models and institutional arrangements between countries, regions and stakeholders. In particular cryopreservation programmes and conservation of transboundary breeds may require new funding mechanisms and/or institutional arrangements. When designing programs for conservation of animal genetic diversity, not only technical criteria are relevant, but also legal, sanitary, socio-economic and institutional issues. In this context a range of future use options (e.g. research, breeding, etc.) should be considered as part of the development of collaborative models between countries.

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